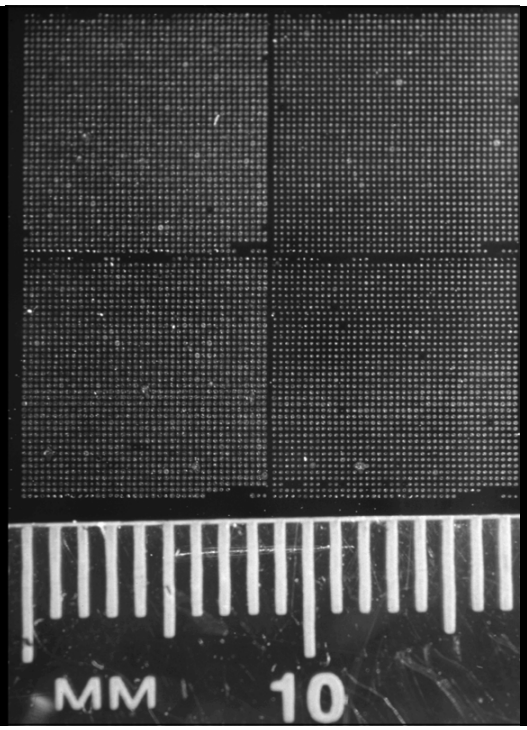
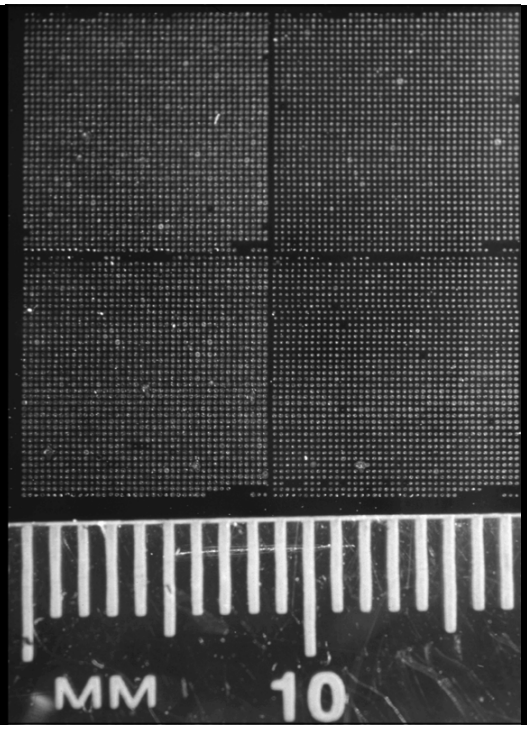
Genome Sequence Shows Few Differences Between Humans, Worms



Saccharomyces cerevisiae

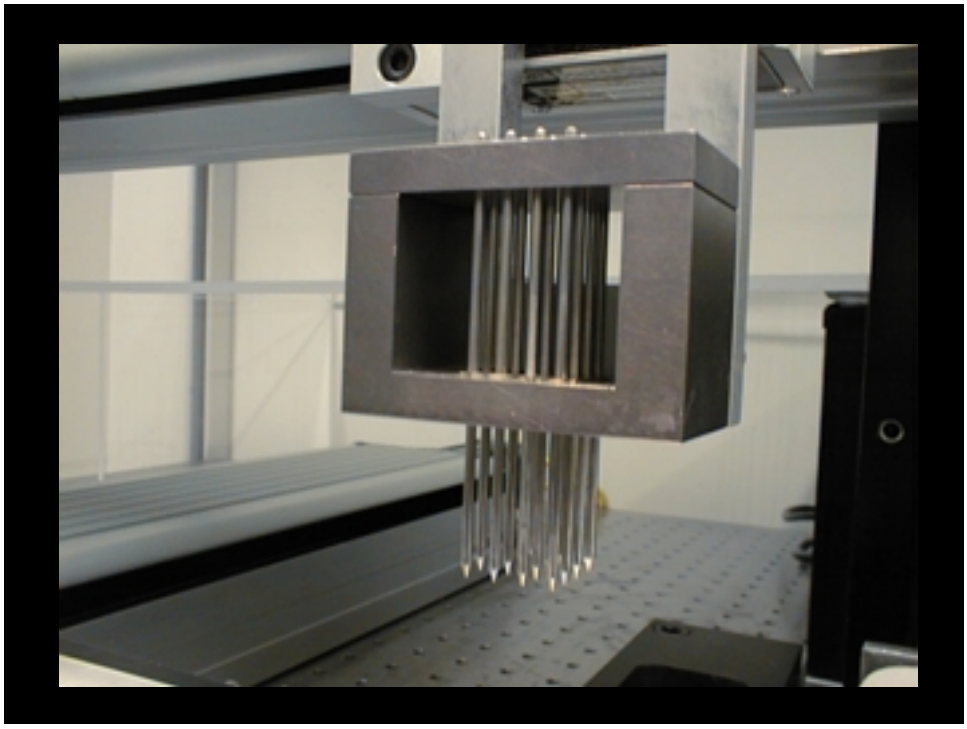
- ~12 M genome; sequencing completed 1996
- ~6,200 ORFs
- When genome sequence completed only ~35% of genes had reasonable functional annotation

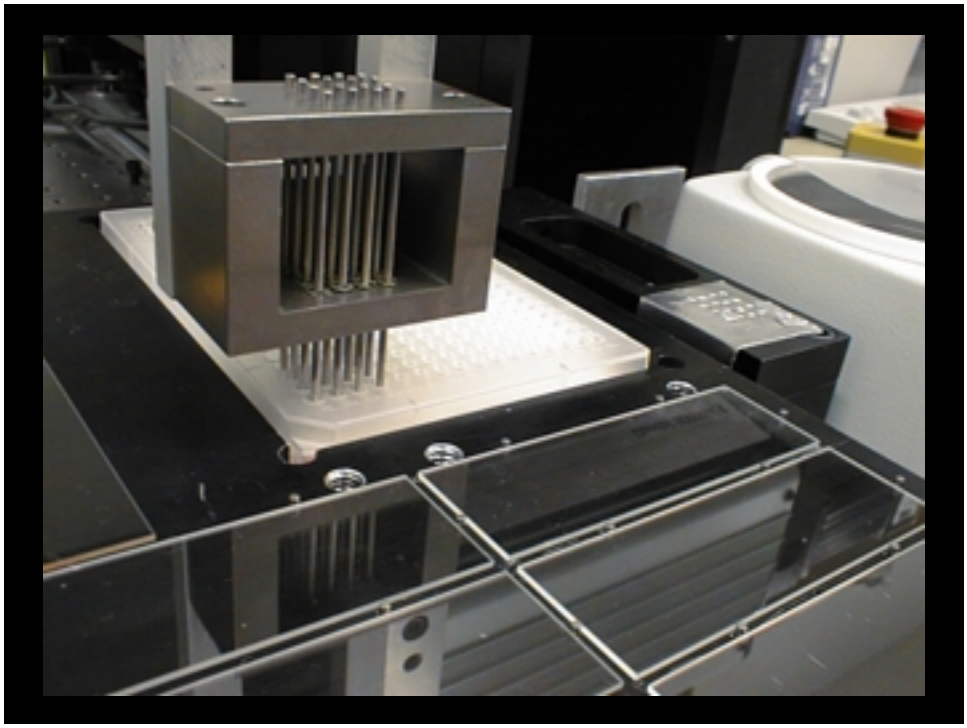


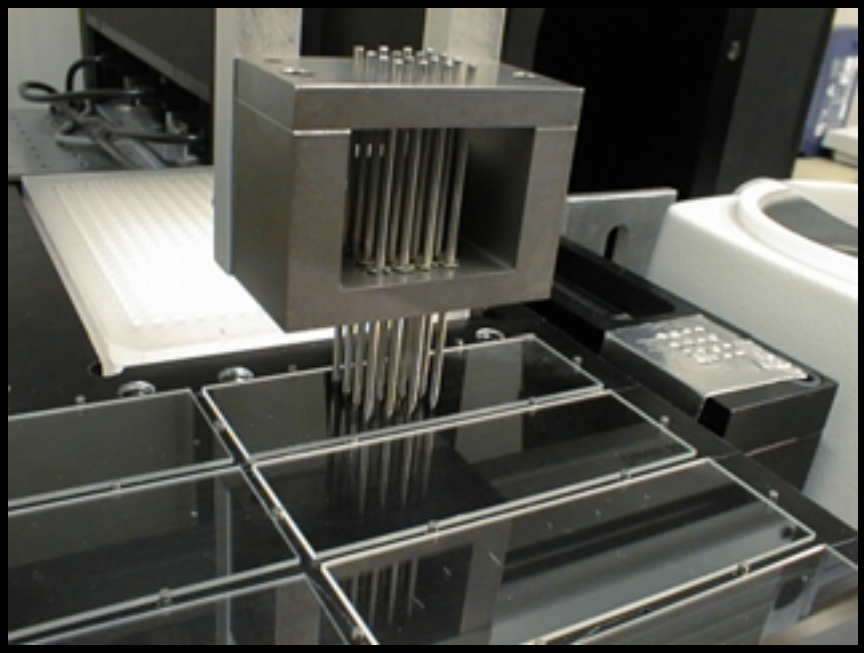
Spotted DNA Arrays

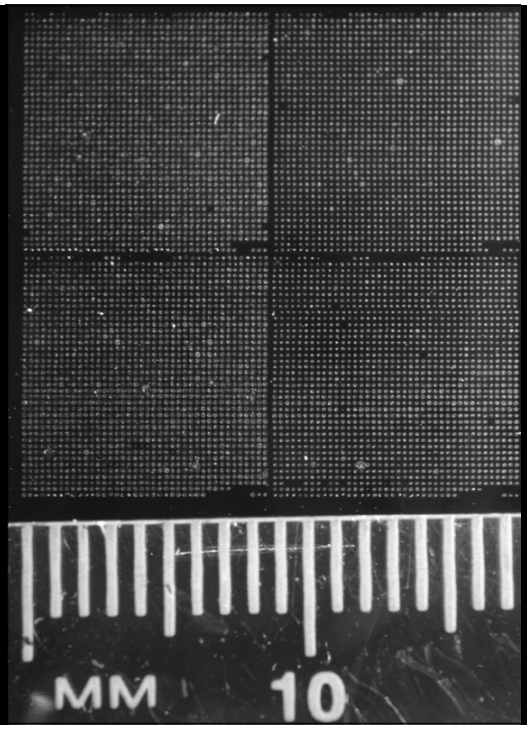
Pre-synthesized DNA (e.g. from PCR or oligo synthesis)

Robotically deposited on treated glass microscope slides in regular array









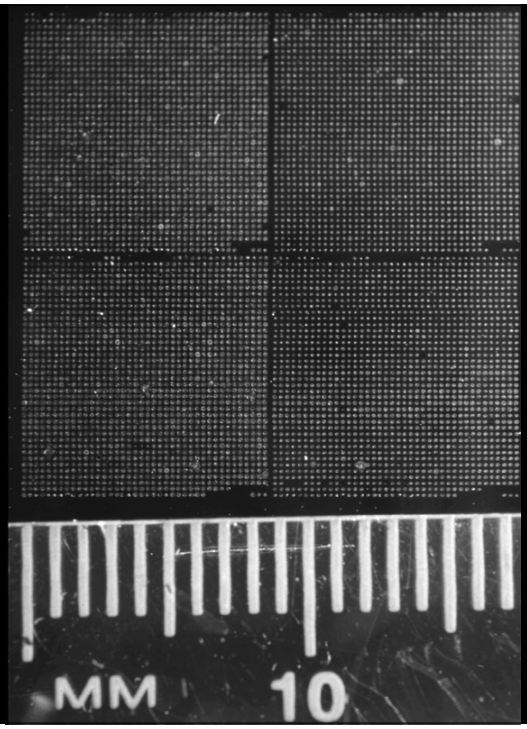
Genome-wide Templates for Hybridization Assays

Examples of spotted material:

All ~6,200 open reading frames from *Saccharomyces cerevisiae*

Human cDNAs

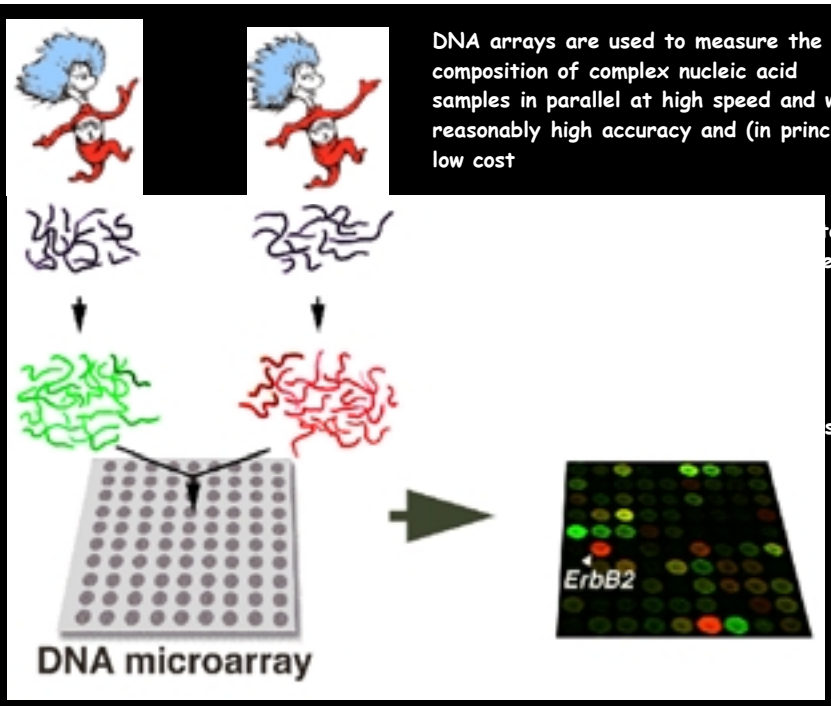
Synthetic 70-mers from coding



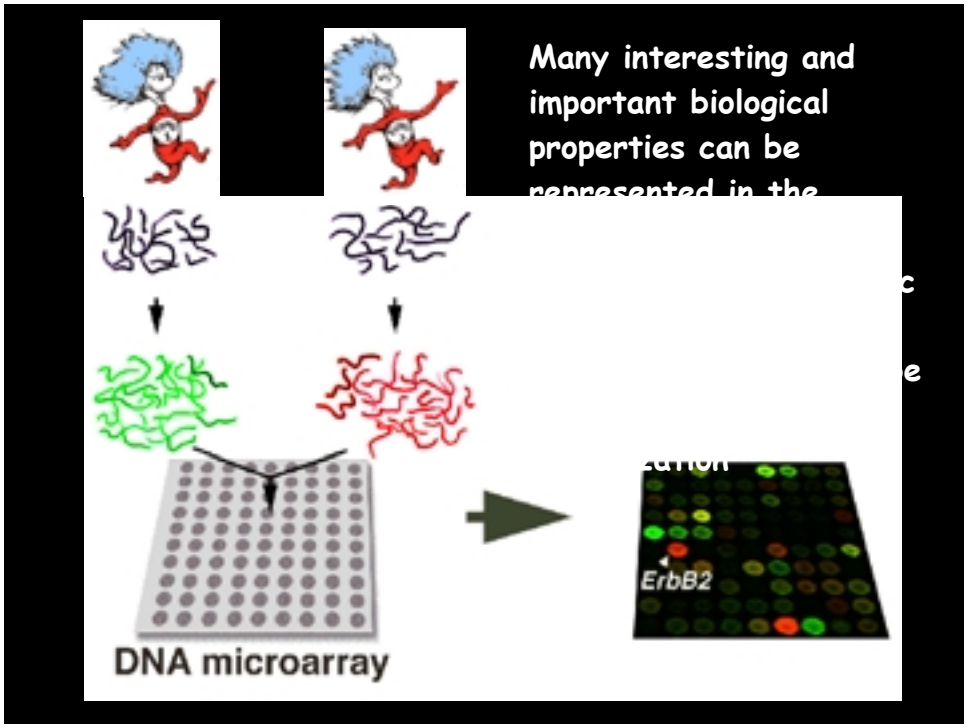
Spotted DNA Arrays

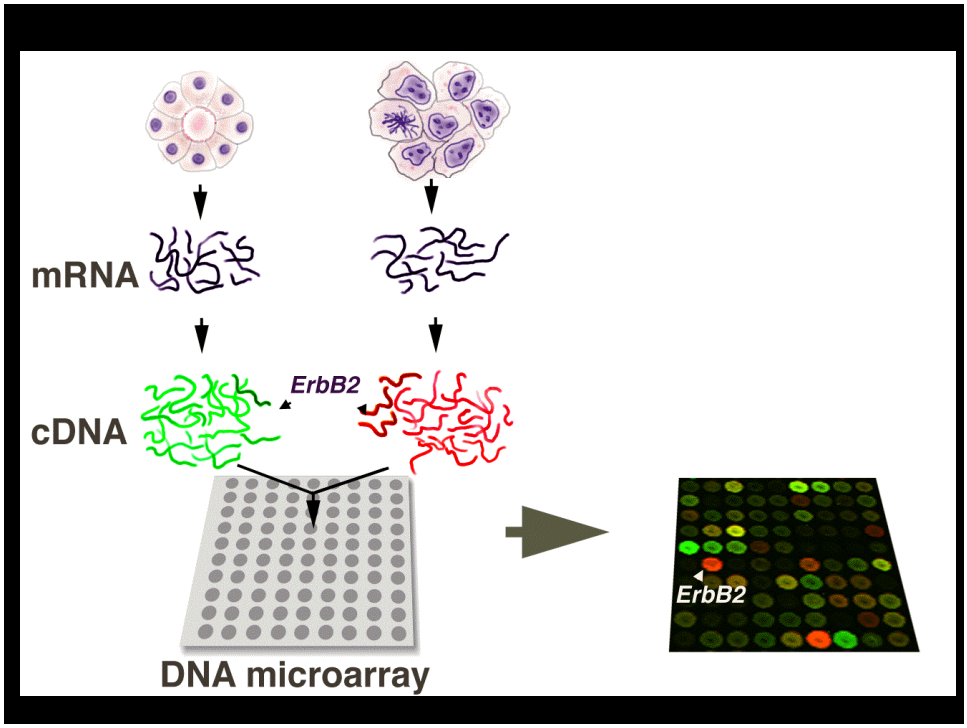
Spots can now be printed with center to center spacing of less than 100um, allowing for more than 150,000 spots to be printed on a standard glass slide.

A good robot can now print 50,000 spots on 200 slides in 24



DNA arrays are used to measure the composition of complex nucleic acid samples in parallel at high speed and with reasonably high accuracy and (in principle) low cost

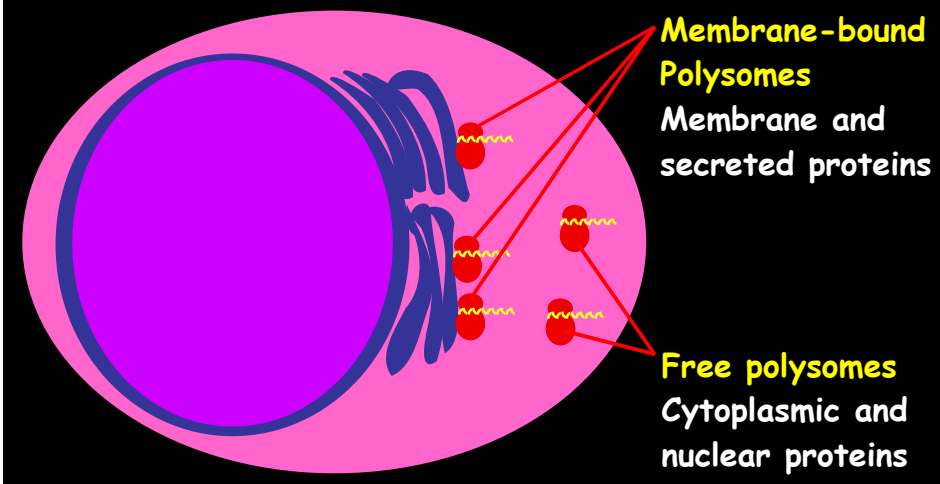

Many interesting and important biological properties can be
ErbB2
DNA microarray

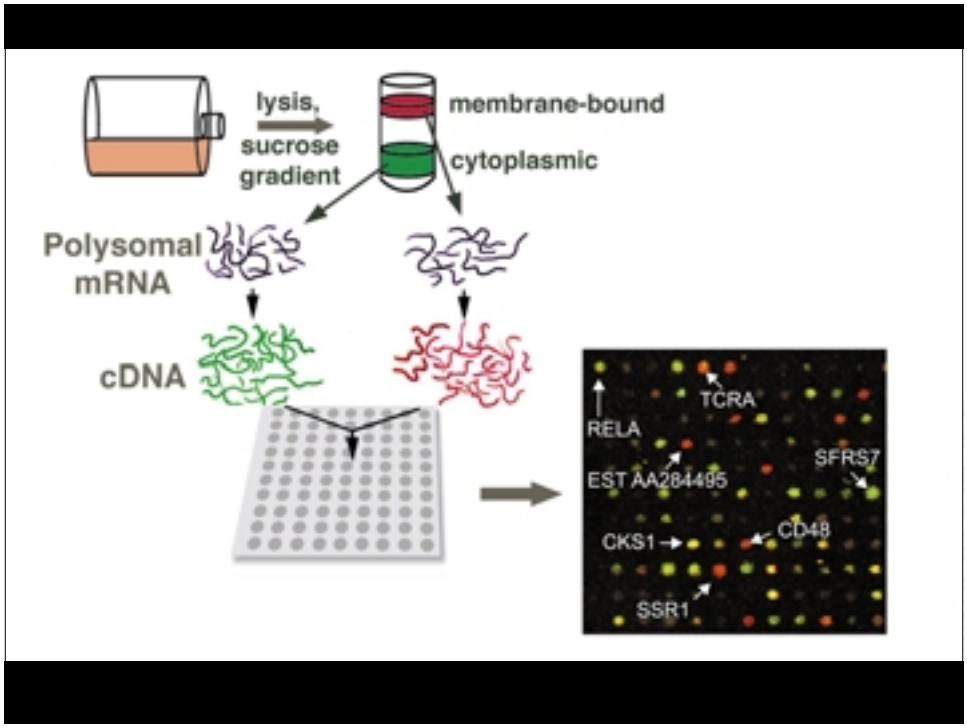

mRNA
cDNA
ErbB2
ErbB2
DNA microarray

Other Properties of Biological Systems and Biomolecules That Can Be Studied By Array Hybridization

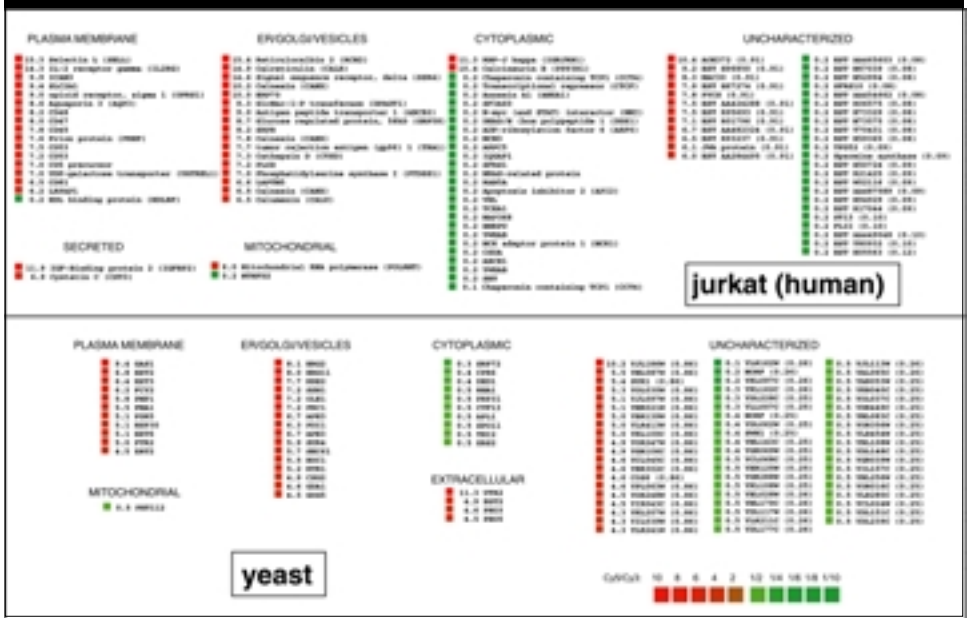
- Transcript Abundance
- Karyotype/DNA copy number
- Identity by descent/Genetic Mapping
- Translation, Transcription, Message Decay Rates
- Sub-cellular localization of transcript/gene product
- *In vivo* binding distribution and *in vitro* binding affinities of DNA binding proteins

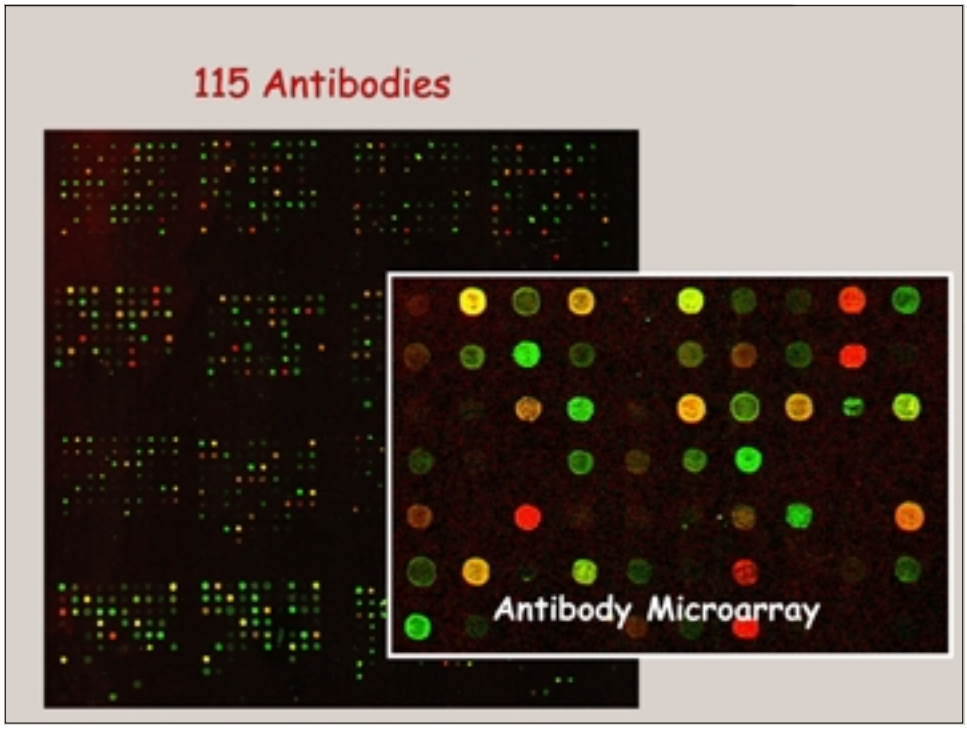
Use of cDNA Microarrays to Identify Genes Encoding Membrane and Secreted Proteins

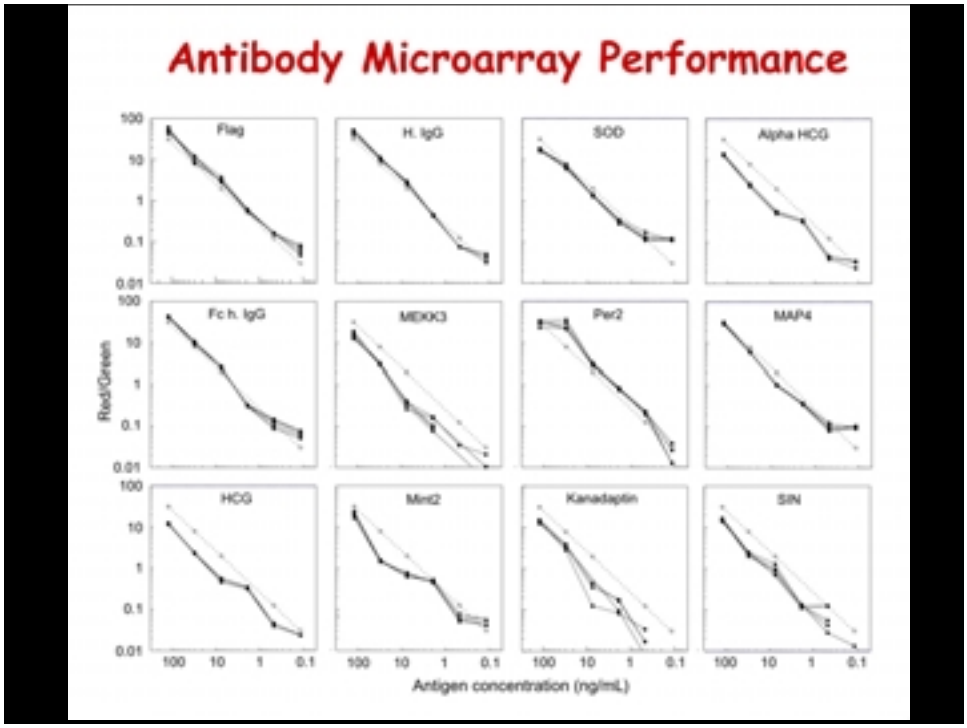




Results of MBP Array Analyses




115 Antibodies
Antibody Microarray


Antibody Microarray Performance
Flag
H. IgG
SOD
Alpha HCG
Fc h. IgG
MEKK3
Per2
MAP4
HCG
Mnt2
Kanadaptin
SIN
Red/Green
Antigen concentration (ng/mL)

The Dynamic Genome

Every individual of every species has, to a first approximation, only one genome, and with this essentially static genome:

Unicellular organisms can survive, grow and reproduce in rapidly and extremely variable environmental conditions.

Multicellular organisms are capable of producing cells and tissues with dramatically different properties.

Evolution of Gene Regulation

Evolution ensures that coding genes make proteins with the proper molecular properties: enzymatic activity, binding, etc….

Equally importantly, natural selection has also proceeded to ensure that these proteins are made and function when, where and in the proper form and amounts required, and that they are not made when their presence would be deleterious, or in unnecessary amounts that would waste cellular energy.

Evolutionary Logic Links Gene Expression and Gene Function

The evolutionary logic that dictates that genes be made only when and where needed implies a direct connection between a gene's pattern of expression and its function

Evolutionary Logic Links Gene Expression and Cellular State/Phenotype

The important role of gene regulation in development and cellular responses means that the particular set of genes a cell or collection of cells is expressing at any moment can tell us a great deal about its history, environment, internal state and future.

Gene expression provides a window onto two properties of biological systems that we are extremely interested in studying

Gene Function

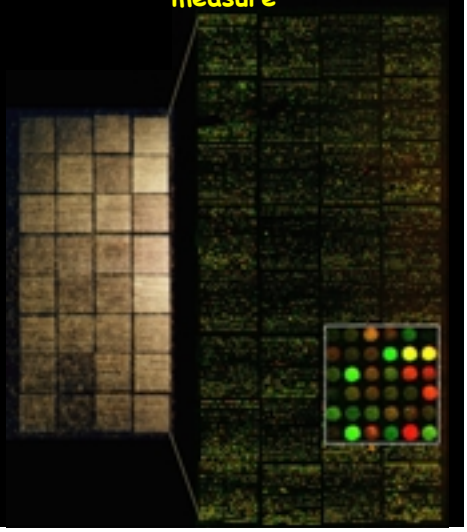
↕

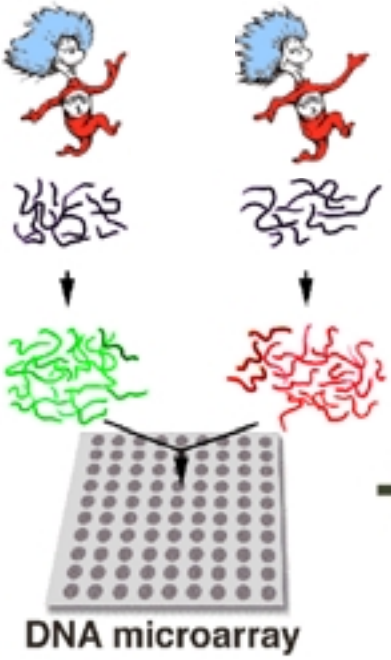
Gene Expression

↕

State/Phenotype

Unlike gene function or the physiological state of cells, tissues and organisms, gene expression is now easy to measure





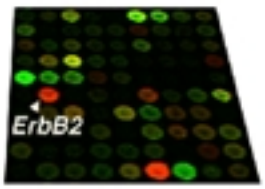
DNA microarray

DNA arrays are used to measure the composition of complex nucleic acid samples in parallel at high speed and with reasonably high accuracy and (in principle) low cost

Two-color comparative hybridization to arrays measures relative abundance of each nucleic acid represented on the array

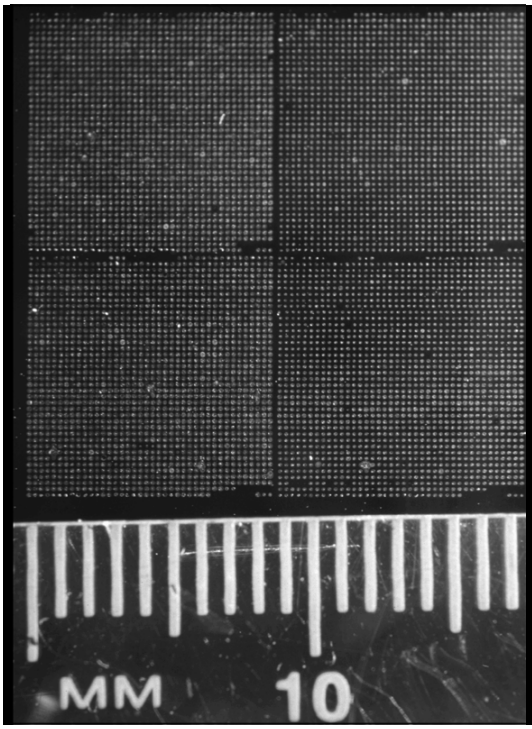
Accurate (~25% error)

Sensitive (detect 1.5x changes at less than 1ppm)

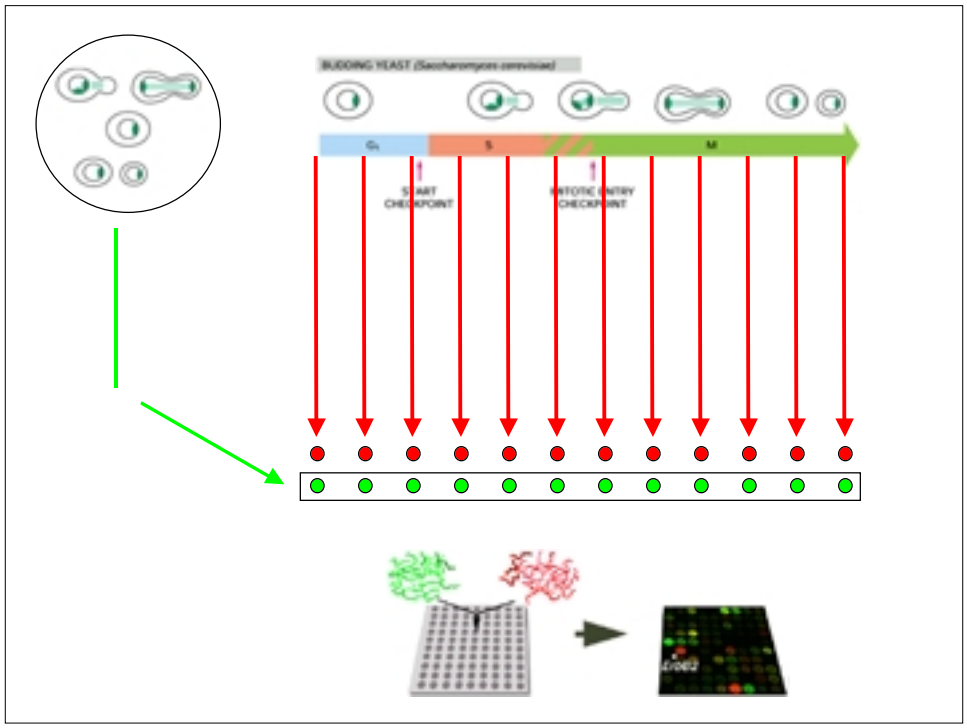


Saccharomyces cerevisiae

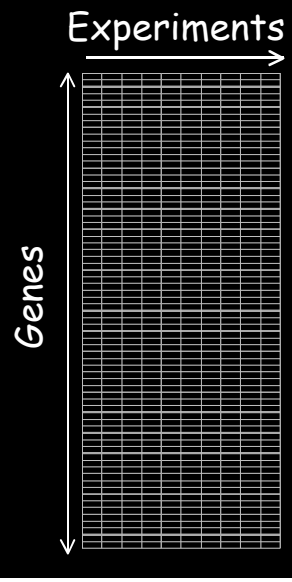
- Unicellular fungus with extensive commercial importance
- ~12 M genome; sequencing completed 1996
- ~6,200 ORFs
- Despite extensive genetic, molecular and biochemical analysis, when genome sequence completed only ~35% of genes had reasonable functional annotation



DNA microarrays with elements representing every identified open reading frame – either in the form of PCR amplified ORFs or synthetic oligonucleotides - have been available for 5 years and are in fairly wide use



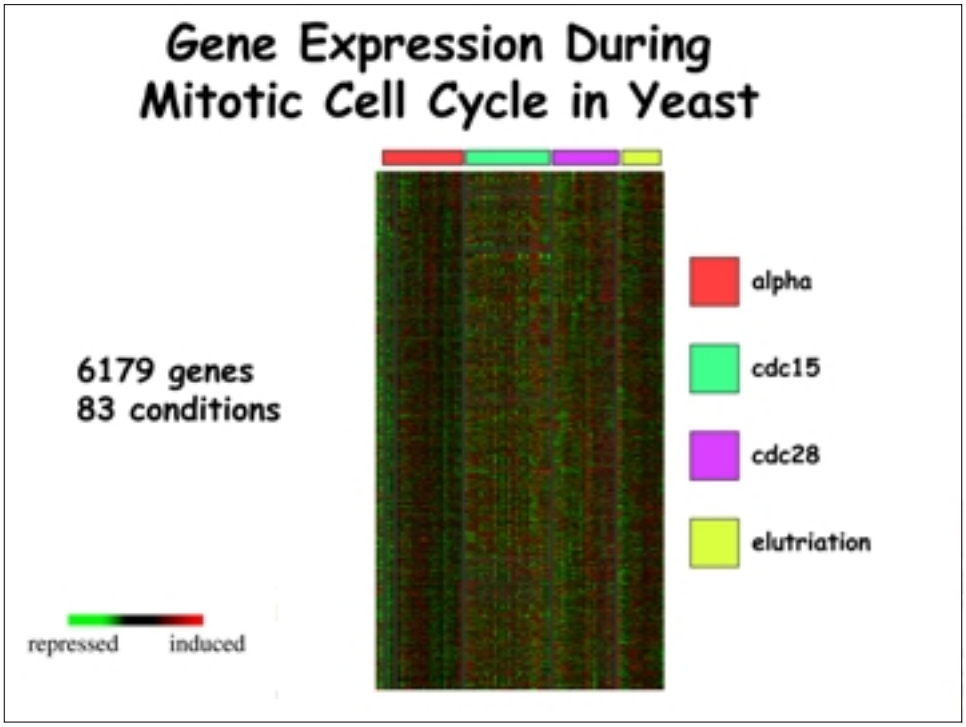
The Principle Challenge in Experimental Genomics is Making Biological Sense of the Data



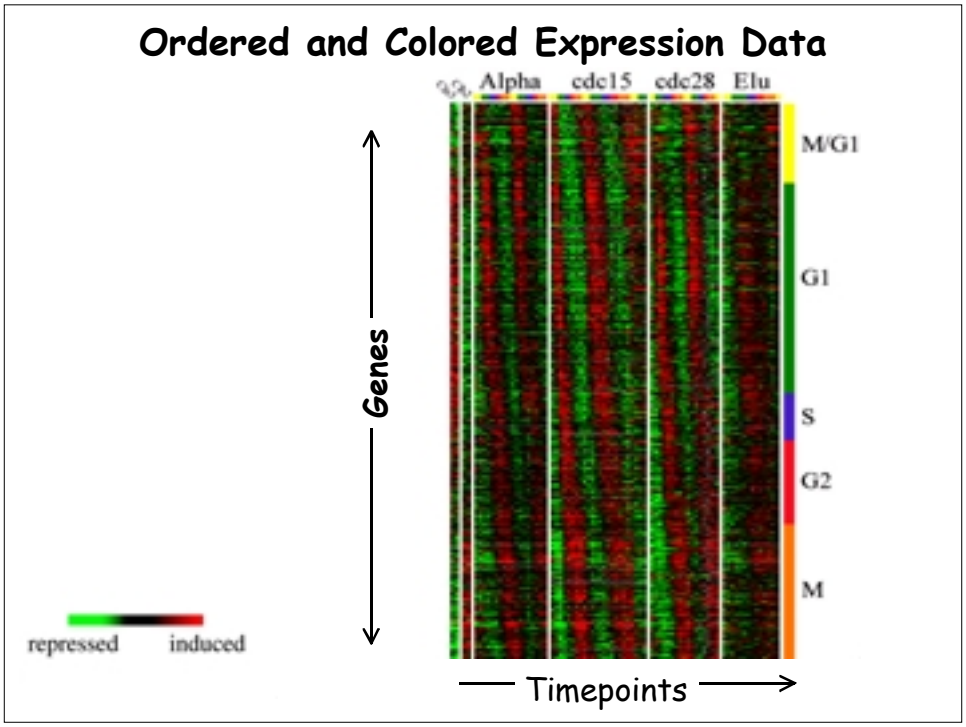
Saccharomyces cerevisiae whole genome ORF arrays

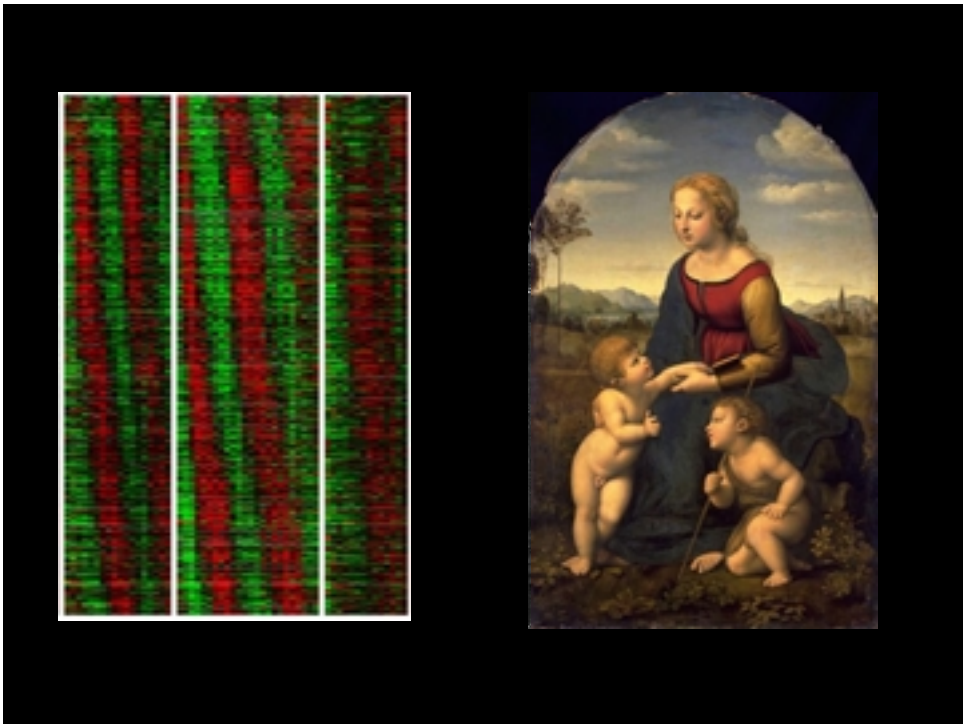
~6200 genes
~6000 conditions
> 36 million observations

Gene Expression During Mitotic Cell Cycle in Yeast



Ordered and Colored Expression Data

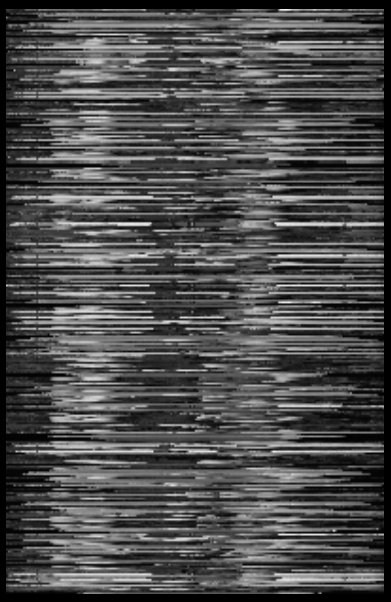


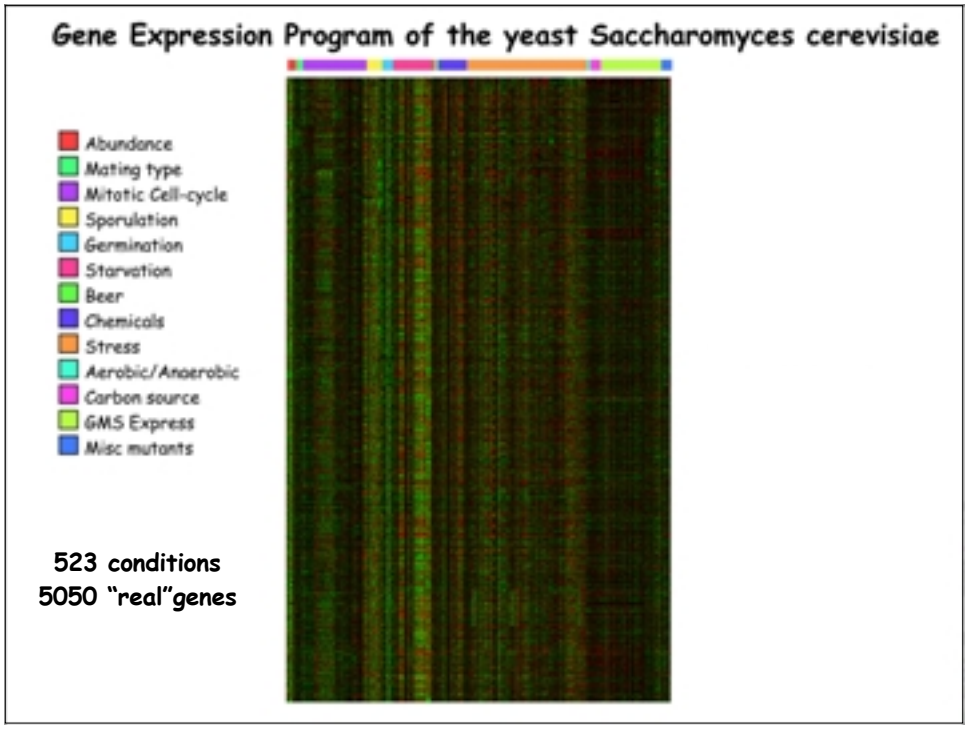


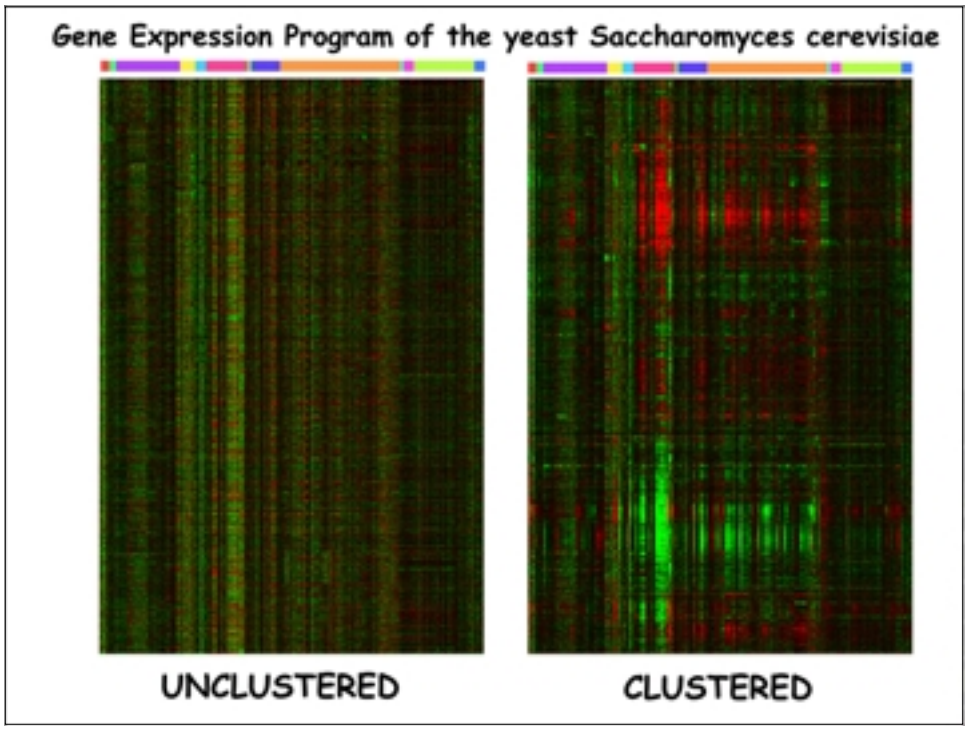
How Do We Make Biological Sense of Complex Gene Expression Datasets

A corollary of this logical relationship between gene expression and gene function is that genes with similar function should have similar patterns of gene expression.

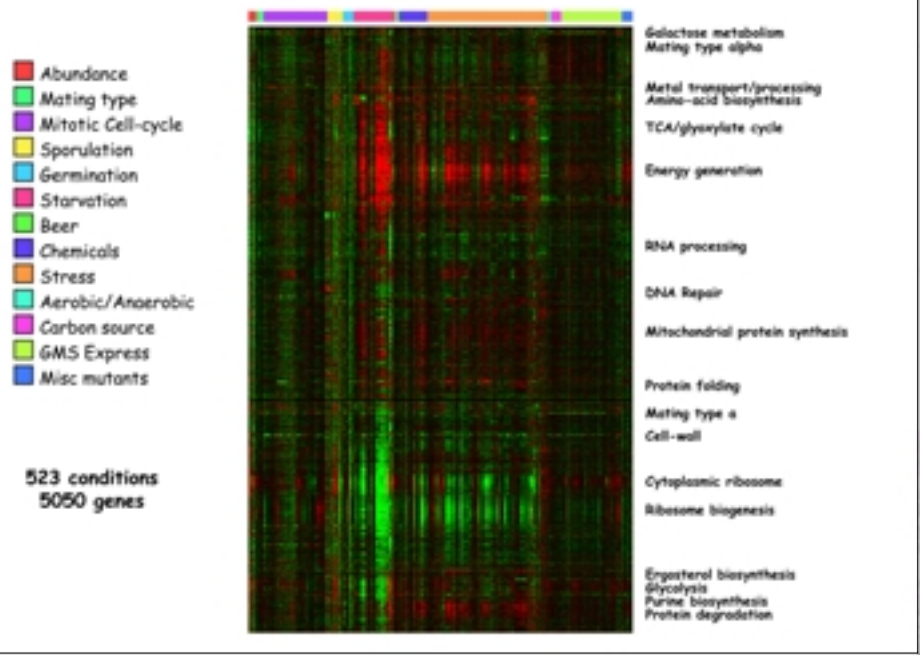
To the extent that this is true, this property can be used to impart a logical and biologically meaningful order to complex gene expression data




Gene Expression Program of the yeast Saccharomyces cerevisiae
Abundance
Mating type
Mitotic Cell-cycle
Sporulation
Germination
Starvation
Beer
Chemicals
Stress
Aerobic/Anaerobic
Carbon source
GMS Express
Misc mutants
523 conditions
5050 "real"genes

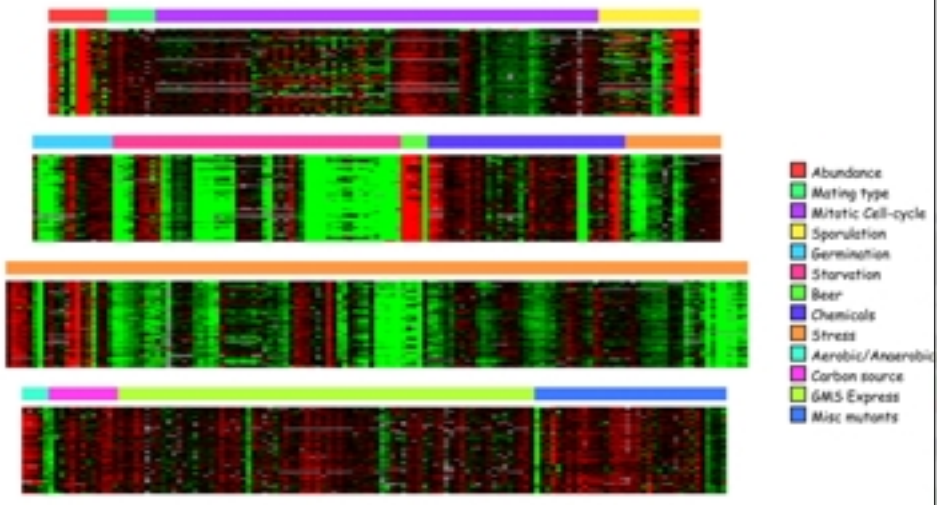

Gene Expression Program of the yeast Saccharomyces cerevisiae
UNCLUSTERED
CLUSTERED

Gene Expression Program of the yeast Saccharomyces cerevisiae



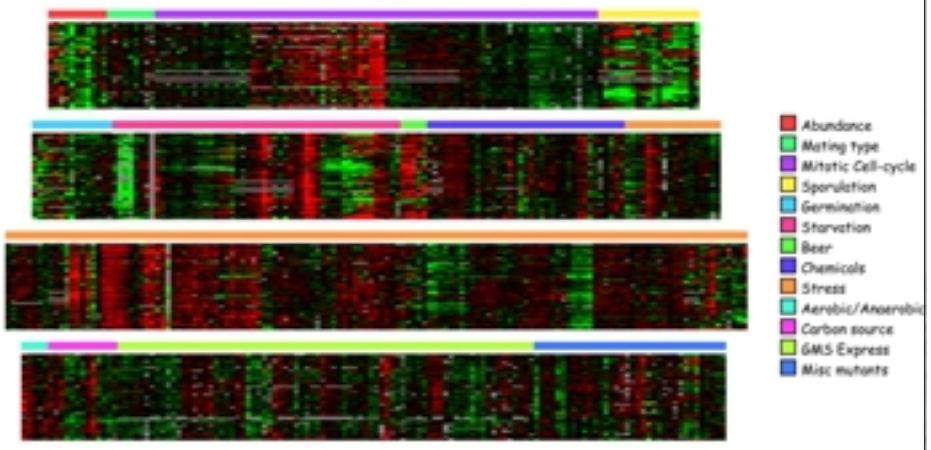
Cytoplasmic Ribosome Cluster

Cluster contains virtually all subunits of cytoplasmic ribosome



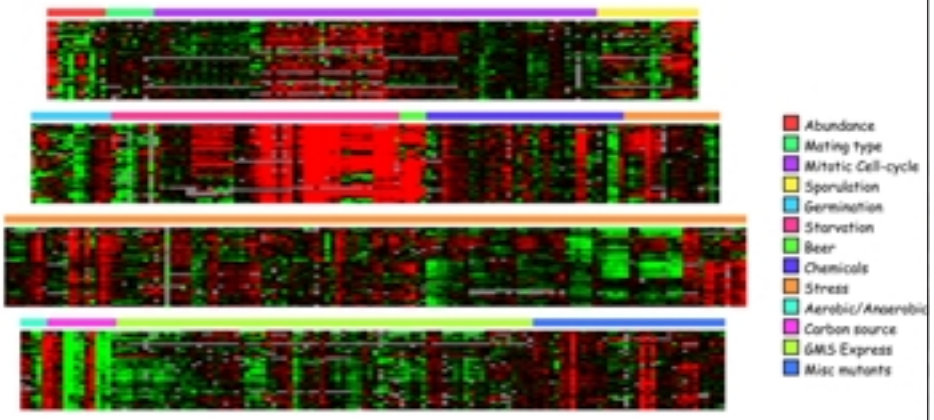
Mitochondrial Ribosome Cluster

Contains most known mitochondrial ribosome components
plus 8 proteins with no annotated function,
3 with homology to bacterial ribosomal proteins



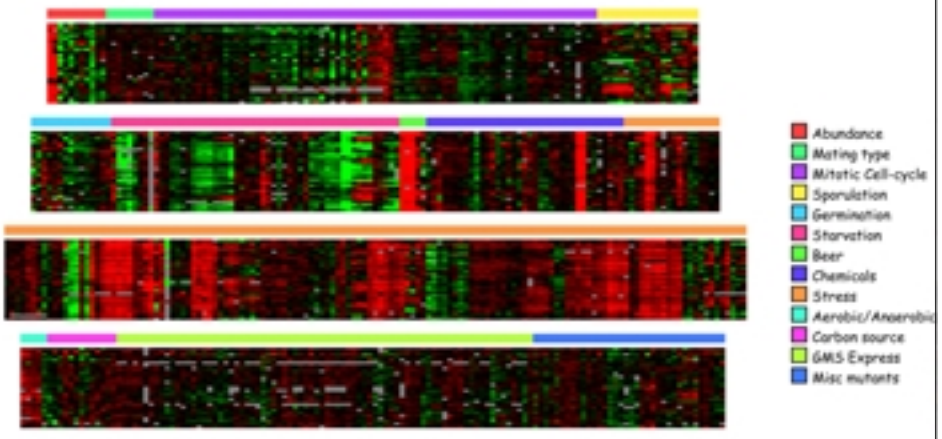
Energy Generation Cluster

Oxidative Phosphorylation and ATP Synthesis

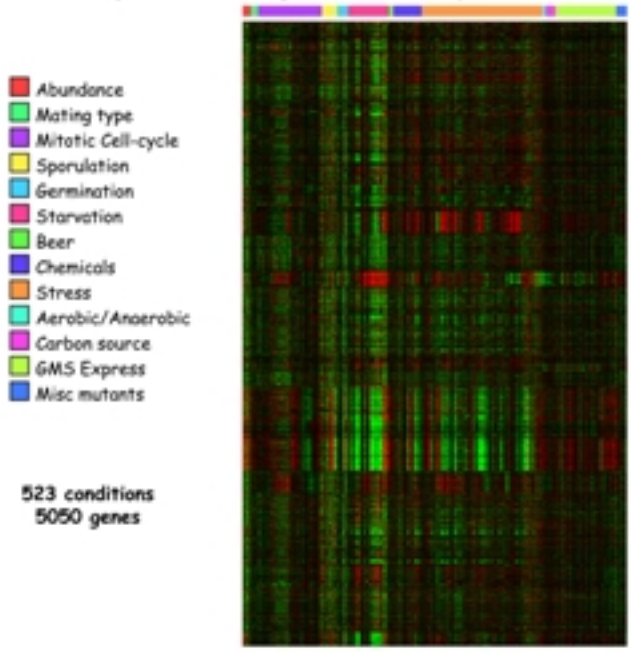


Proteasome Cluster

Contains virtually all known components of proteasome
(20s and 26s subunits)

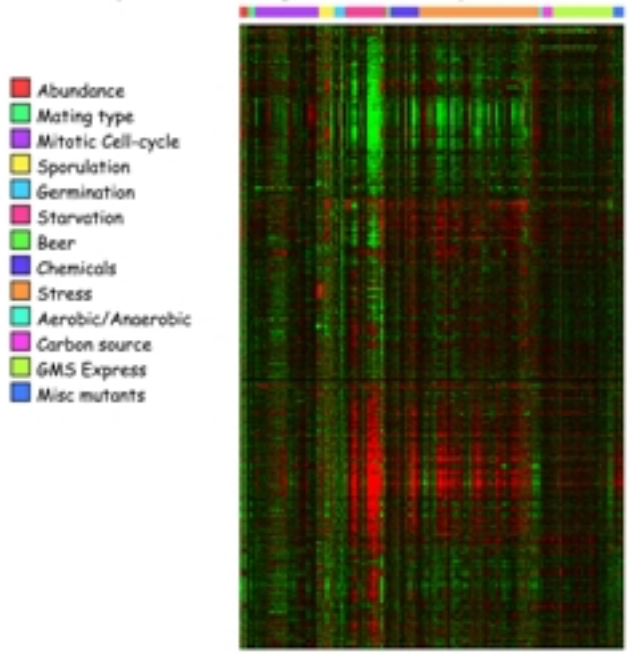


Gene Expression Program of the yeast Saccharomyces cerevisiae



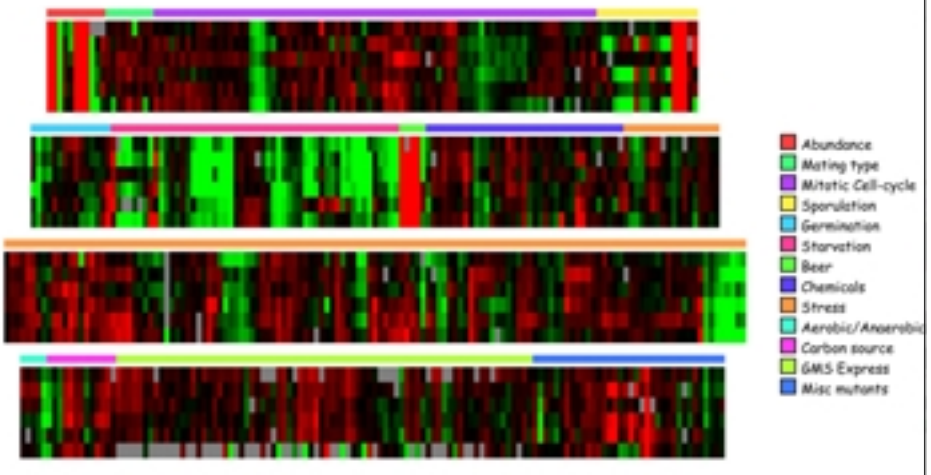
All known multisubunit complexes

Gene Expression Program of the yeast Saccharomyces cerevisiae



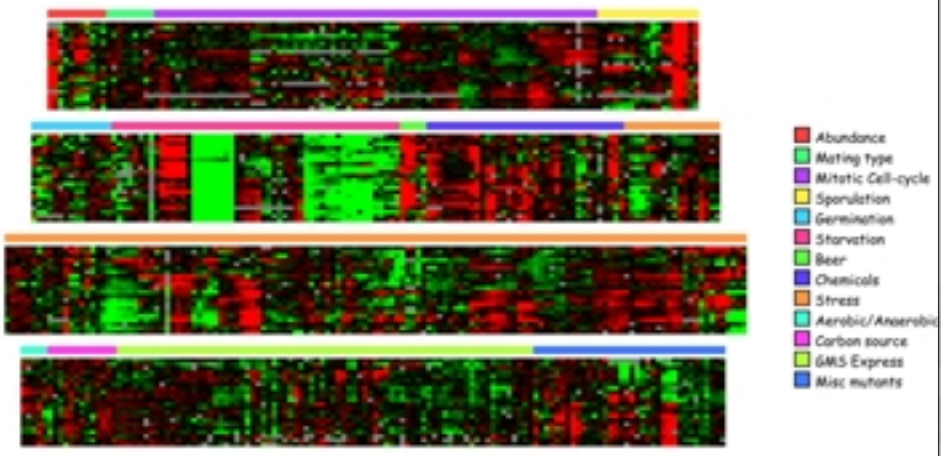
Proteins not known to be part of multisubunit complexes

Glycolysis Cluster



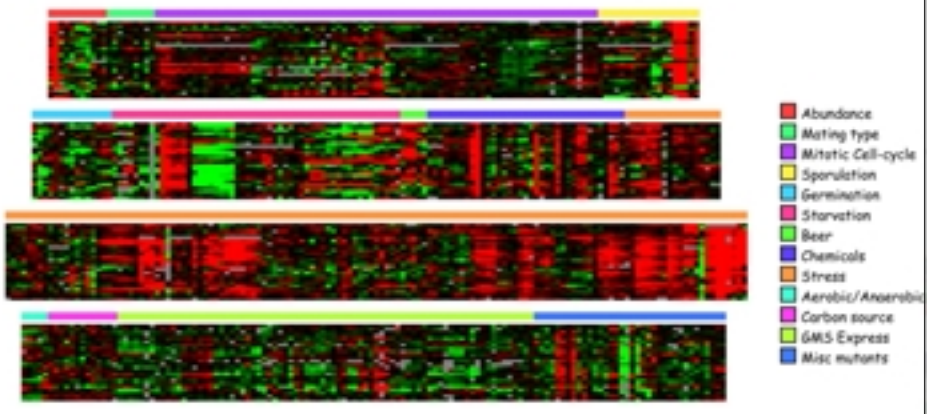
Amino Acid and Purine Biosynthesis

Lue, Ile, Val, Lys
Purine, His



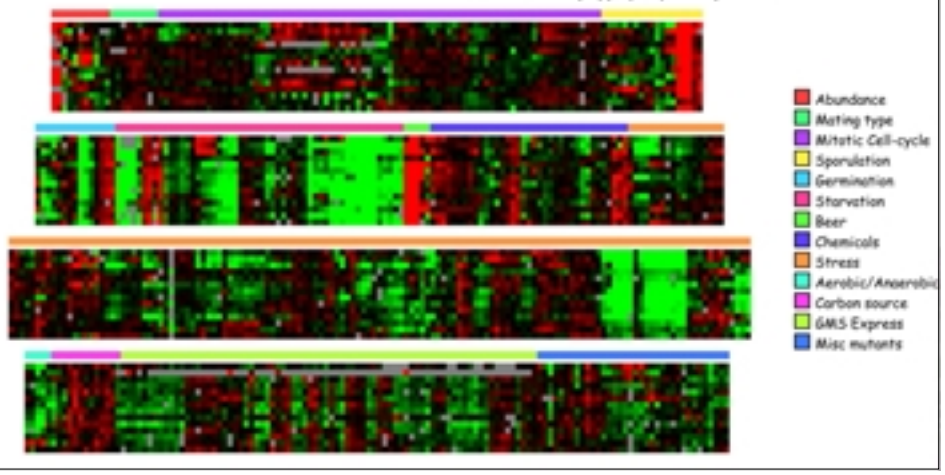
Amino Acid and Purine Biosynthesis

Arg,Trp + misc



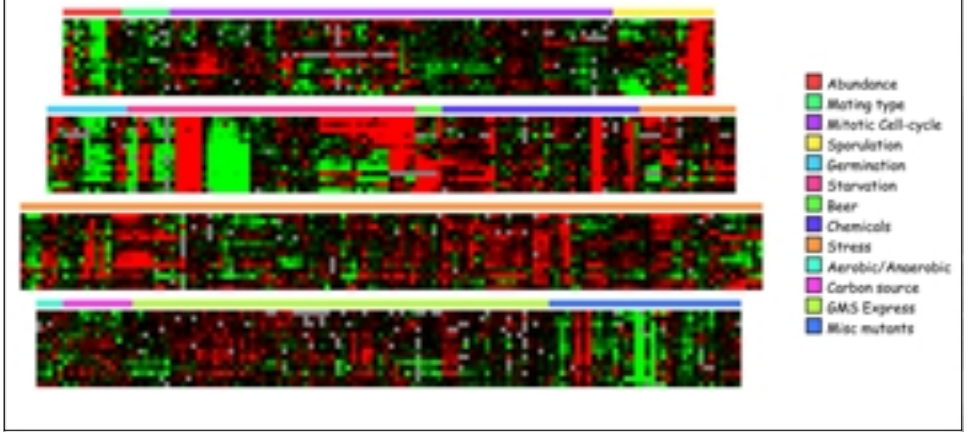
Ergosterol Biosynthesis Cluster

ERG5	C-22 sterol desaturase
CYB5	cytochrome b5
ERG3	C-5 sterol desaturase
ERG12	mevalonate kinase
ERG10	acetoacetyl CoA thiolase
ANB1	translation initiation factor
ERG2	C-8 sterol isomerase
MVD1	mevalonate pyrophosphate decar
ERG13	3-hydroxy-3-methylglutaryl coenzyme A synthetase
ACS2	acetyl-coenzyme A synthetase
ERG1	squalene monooxygenase
ERG26	sterol C-3 dehydrogenase
ERG6	delta-24 sterol C-methyltransferase
ERG20	farnesyl-pyrophosphate synthetase



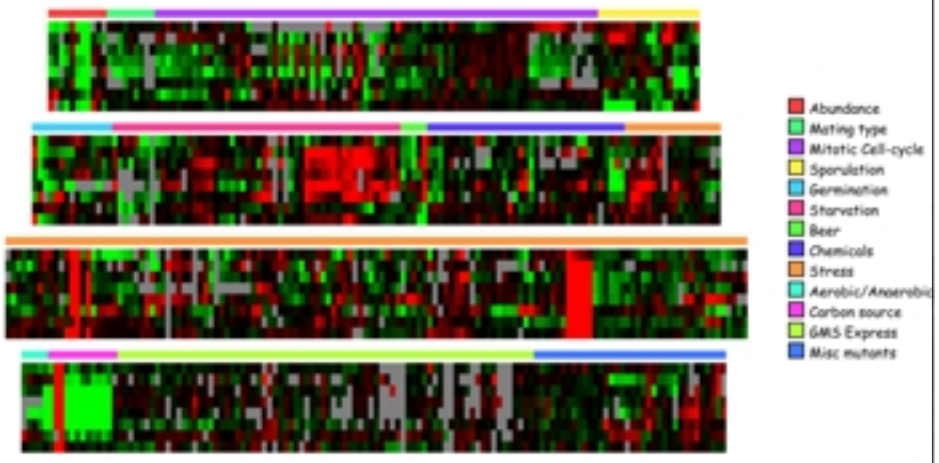
Methionine Biosynthesis Cluster

RAD59	
MET32	transcription factor
MET28	transcriptional activator
MET2	homoserine O-acetyltransferase
SUL2	sulfate permease
MET1	siroheme synthase
MET16	3'-phosphoadenylylsulfate reductase
MET5	sulfite reductase
MET10	sulfite reductase subunit
MET3	sulfate adenylyltransferase
MET14	adenylylsulfate kinase
MET17	O-acetylhomoserine sulfhydrylase
MET13	methylenetetrahydrofolate reductase
MET6	homocysteine methyltransferase
SER3	3-phosphoglycerate dehydrogenase
MET22	3'2'5'-bisphosphate nucleotidase

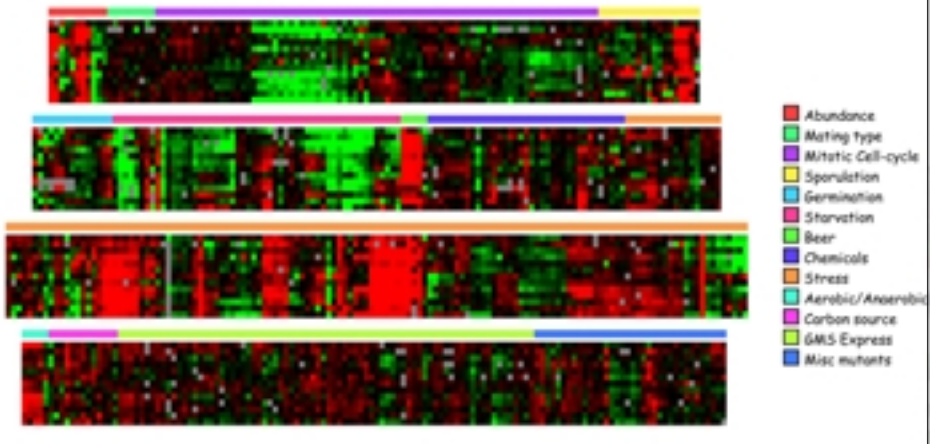


Galactose Utilization Cluster

GAL1,2,3,7,10,80 plus
vessicular transport protein of unknown function

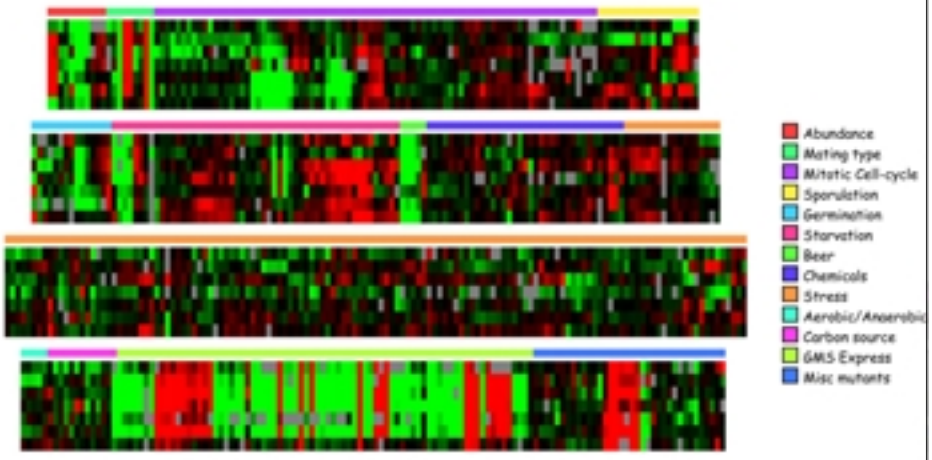


Protein Folding Cluster



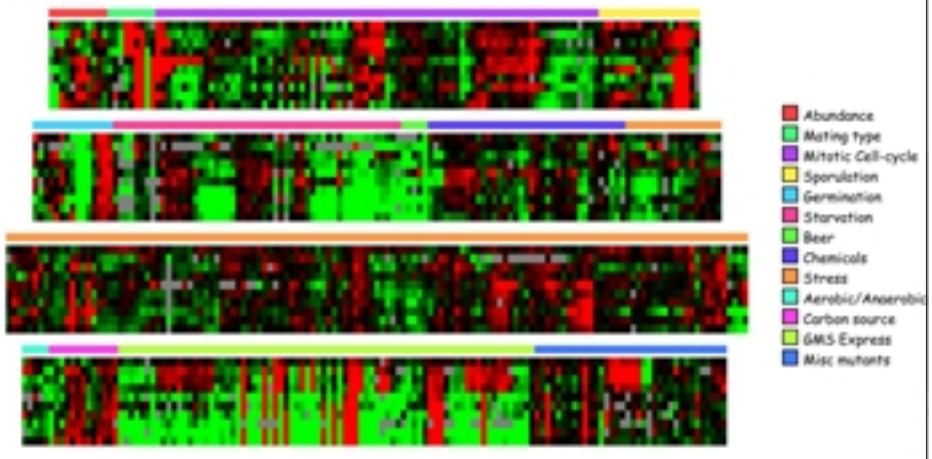
Mating-type Alpha Cluster

MATALPHA1	alpha-specific gene activator
YLR040C	unknown
STE3	a-factor receptor
MFALPHA1	alpha factor
MFALPHA2	alpha factor
SAG1	alpha-agglutinin
LIF1	unknown

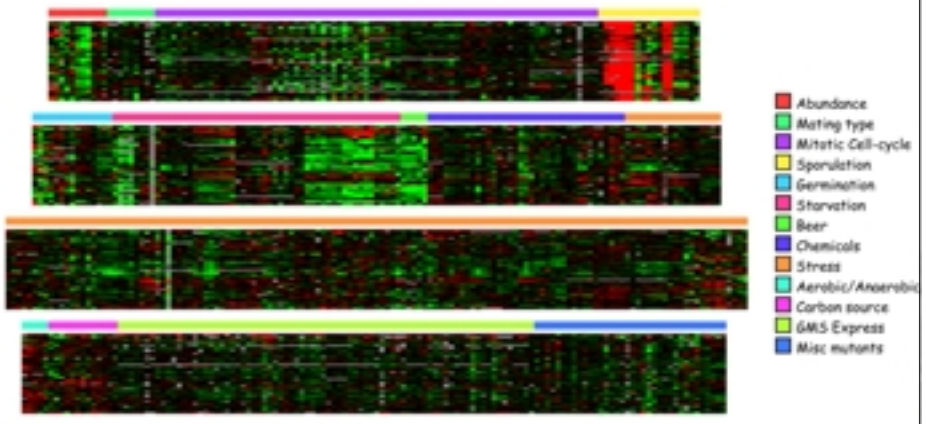


Mating-type a cluster

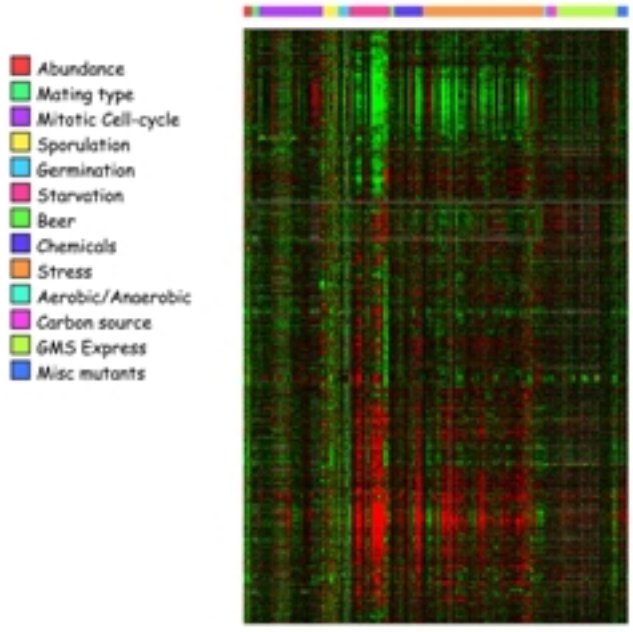
KAR4	pheremone transcription
SST2	alpha-factor desensitization
AGA1	a-agglutinin anchor subunit
FUS1	fusion; SH3 domain protein
MFA1	a-factor precursor
AGA2	a-agglutinin binding subunit
MFA2	a-factor precursor
BAR1	alpha-factor degradation
STE2	alpha-factor receptor
STE6	a-factor exporter



Sporulation Cluster



Gene Expression Program of the yeast Saccharomyces cerevisiae



Genes of Unknown or Poorly Characterized Function

Reading Genome Sequences

Great progress has been made in reading the protein coding information contained in genomes: identifying the location and structure protein coding genes, and of using the amino acid sequences to predict gene function and 3D structure

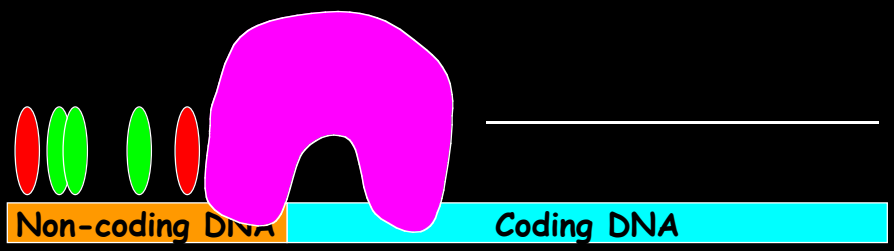
Reading Genome Sequences

Comparably little progress has been made in reading the non-coding content of genomes, especially information that encodes when, where and under which conditions genes will be expressed.

Reading Genome Sequences

The sequencing of the human genome was accompanied by the prediction of ~35,000 protein coding genes by two independent research teams, but essentially no predictions about the likely expression patterns of these genes.

How is regulatory information written in genomes?



Reading cis-Regulatory Code What do we need to know?

Output of system

Temporal, Spatial and Conditional gene expression patterns of all genes

Reading cis-Regulatory Code What do we need to know?

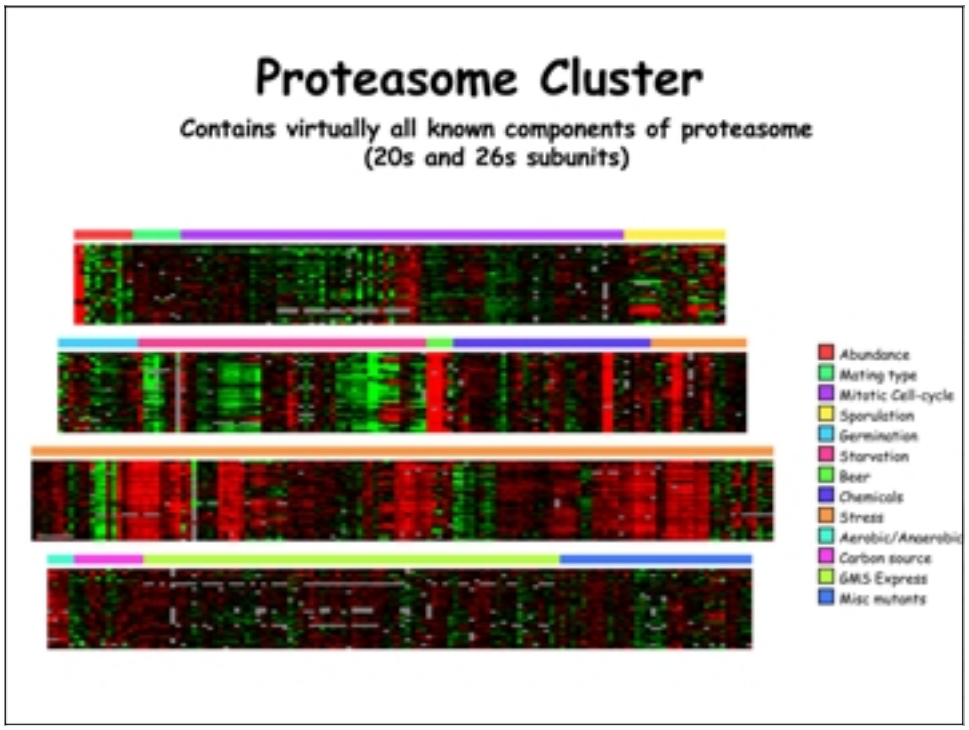
Input to system

- cis-DNA sequence
- *in vitro* binding affinities of transcription factors
- *in vivo* binding distribution of transcription factors
- Evolutionarily conserved non-coding sequences

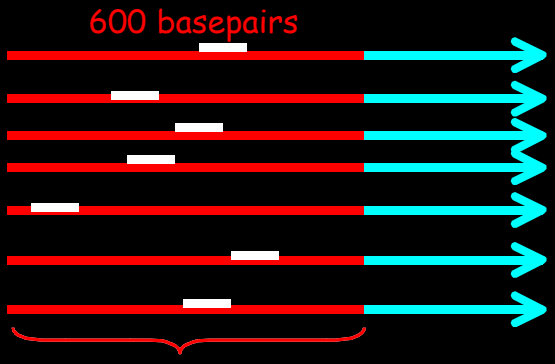
Reading cis-Regulatory Code
What do we need to know?

Input to system

- cis-DNA sequence
- *in vitro* binding affinities of transcription factors
- *in vivo* binding distribution of transcription factors
- Evolutionarily conserved non-coding sequences



Cluster of co-expressed genes
Pattern discovery in regulatory regions



Pattern over-represented in cluster

Possible examples of motif 1 in the training set

Sequence name	strand	Start	Score	Site	
YLR118C	w53	736	10.74	AAATAAAAAT	GTTAGCAAAA GAGAACGAAA
YDL126C	w53	660	17.33	ATGTGAATTC	GGTGGCAAAA AAGGACATAT
YER021W	c53	307	10.44	TTTCTTTGAT	GGCGGCAAAT ACTAATCTTA
YER021W	c53	705	17.07	CTCTTTCTTG	GGTGGCAAAT GTTTTTCGTC
YPR108W	c53	646	15.17	ATCCTCAATG	GGTGGCAAAC TTAACTTATT
YDR427W	w53	560	9.77	TCACGCATCG	TGTTGCAAAT AGCATATACA
YDR427W	w53	692	17.33	GCTTTCATCC	GGTGGCAAAA GTAAGAACAA
YOR157C	w53	643	14.30	AACCCGGCCG	AGTGGCAAAA TACCAAAAAG
YDL097C	w53	688	17.07	TGGTTCCAAG	GGTGGCAAAT GTTGGTAGAA
YOR117W	w53	637	17.33	GCCAATATGT	GGTGGCAAAA ATGAATTAAT
YML092C	w53	681	17.33	AGCTTATTAA	GGTGGCAAAA TTGTCCCCGG
YER012W	w53	659	17.33	AAATCTTTAC	GGTGGCAAAA AATAAAGAAA
YLR387C	w53	709	15.03	GTCACAAGAC	GGTGGCAACA CAGTTGTACA
YDR394W	w53	625	17.33	CTGAAAGTAA	GGTGGCAAAA TAGTAAATCT
YPR103W	w53	646	17.33	ACAGCAAAAG	GGTGGCAAAA CGAAGAATAG
YJL001W	w53	683	17.33	AACGGAATCC	GGTGGCAAAA AAGGGAAAAG
YFR050C	c53	689	17.07	ACGTATTTAA	GGTGGCAAAT TCTAAACCAA
YFR052W	c53	703	17.07	AATTTCAATG	GGTGGCAAAT AATGTGTAGA
YGL011C	w53	446	10.40	TTAGGATTGT	GGTGGTAAAA CTTGTAAAAT
YGL011C	c53	692	17.07	CTATCCTTAC	GGTGGCAAAT TGGTGTGTGC
YKL145W	c53	645	17.33	TTAGTTTTCG	GGTGGCAAAA TTCGGTAATC
YGL048C	w53	717	17.33	ATGCGAGCGA	GGTGGCAAAA GTTGTAATAT
YGR135W	w53	642	17.33	TTACCCTGCA	GGTGGCAAAA AATTTATAGA
YGR253C	c53	480	10.28	CTTCAGCAGT	AGTTGCAAAA GTGTCCTTTT
YGR253C	w53	697	17.33	AACCAAGACC	GGTGGCAAAA GAGTAACCTA
YBL041W	w53	714	17.33	AAACTCAAAG	GGTGGCAAAA ACAAGCGGTA
YFR004W	w53	677	17.07	GGACCTCCAA	GGTGGCAAAT TCACAAGAAA
YJL031C	c53	162	11.38	GTGGCTTCTT	GGTGGAAAAT GCAATCAATT
YJL031C	c53	675	13.79	TGCTGTTTAA	TGTGGCAAAT ATAAAAAATT
YMR004W	w53	169	12.36	AAAATTATCA	GGTCGCAAAT CAGATTTGCT
YMR004W	c53	182	10.21	GATAAGTCTT	TGTAGCAAAT CTGATTTGCG
YBR170C	w53	725	14.03	CAGAACGAAG	AGTGGCAAAT AATAGGACAG
YOR059C	w53	727	14.77	ATAAAGAAAG	GGTGGCAACT CTTTACTGCA
YKR014C	w53	168	10.78	AATGTGGATT	TTTGGCAAAT CAATGTTTCT
YJL053W	c53	465	10.08	GACCCAGTTC	TGTGGCCAAT CTTTTTCTTC
YPR107C	w53	557	15.17	ATCCTCAATG	GGTGGCAAAC TTAACTTATT
YGL047W	c53	113	11.64	CGTTGATGAT	GGTGGAAAAA GTTCCTGATT
YGL047W	c53	236	10.04	ACATCGTGGA	TGTTGCAAAA GATATAAACG
YGL047W	c53	610	17.33	ATGCGAGCGA	GGTGGCAAAA GTTGTAATAT

Proteasome Cluster -600 to -1

MEME (www.sdsc.edu/MEME)

RPN4 Subunit of the regulatory particle of the proteasome

Gene Name/Synonyms RPN4; SON1; UFD5; GVM1; D2840; YDL020C

At-a-Glance

Cellular Role	Protein degradation
Biochemical Function	Proteasome subunit
Localization	Nuclear;19S regulatory particle of the proteasome
Mutant Phenotype	Null: viable

mammalian homolog cannot be found in purified proteasomes
has two potential nuclear localization (NLS) sequences
not detectable in the purified proteasome preparation by
direct sequencing or by detection with antibodies

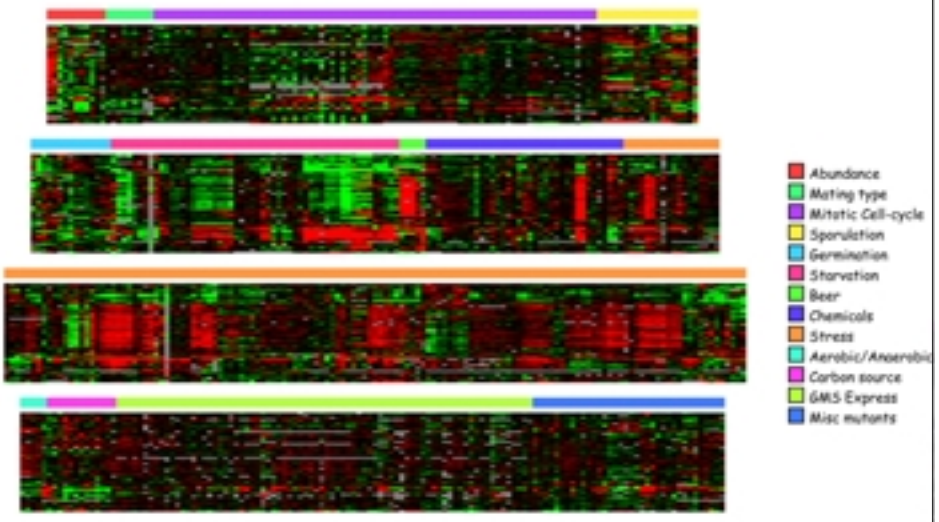
GGTGGCAA is a binding site for RPN4

***FEBS Lett* 1999 Apr 30;450(1-2):27-34**

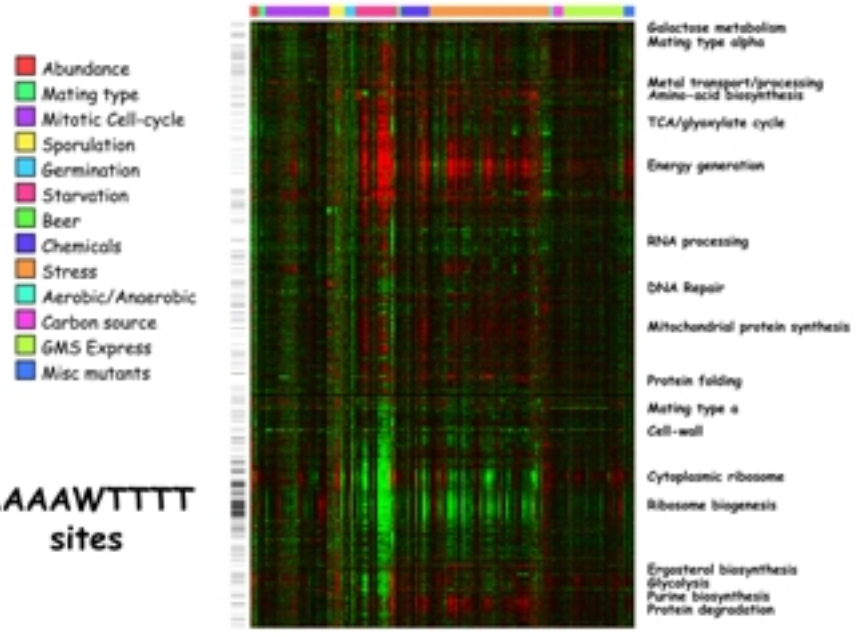
Rpn4p acts as a transcription factor by binding to PACE, a nonamer box
found upstream of 26S proteasomal and other genes in yeast.
Mannhaupt G, Schnall R, Karpov V, Vetter I, Feldmann H
Adolf-Butenandt-Institut der Ludwig-Maximilians-Universitat Munchen, Germany.

We identified a new, unique upstream activating sequence
(5'-***GGTGGCAAA***-3') in the promoters of 26 out of the 32 proteasomal yeast genes characterized to date, which we propose to call proteasome-associated control element. By using the one-hybrid method, we show that the factor binding to the proteasome-associated control element is Rpn4p, a protein containing a C2H2-

Genes with Proteasome Control Element GGTGGCAAA



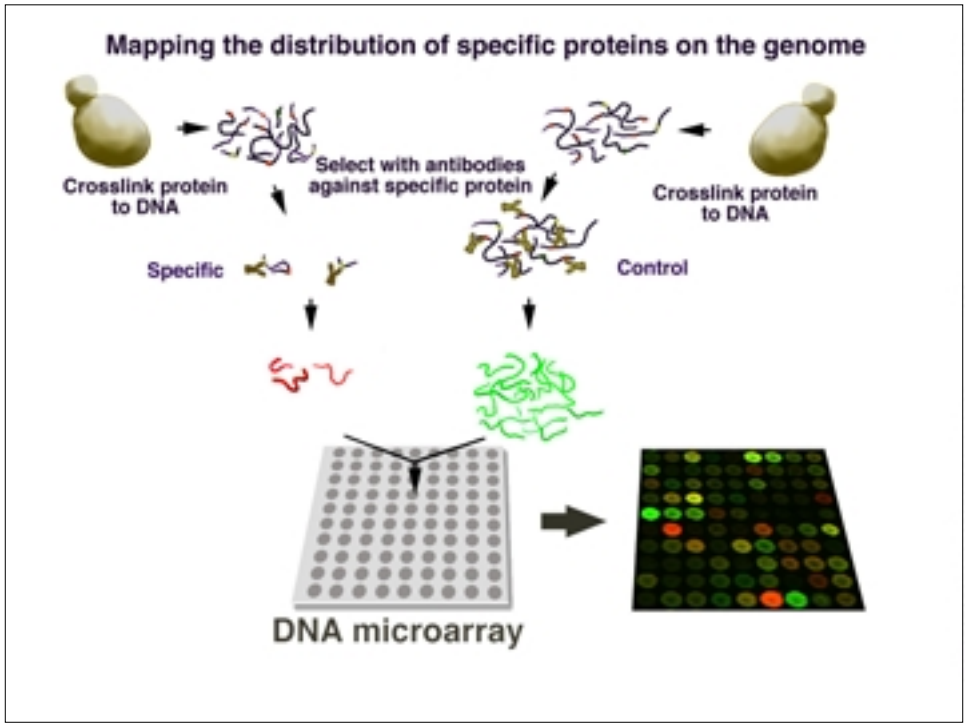
Gene Expression Program of the yeast Saccharomyces cerevisiae

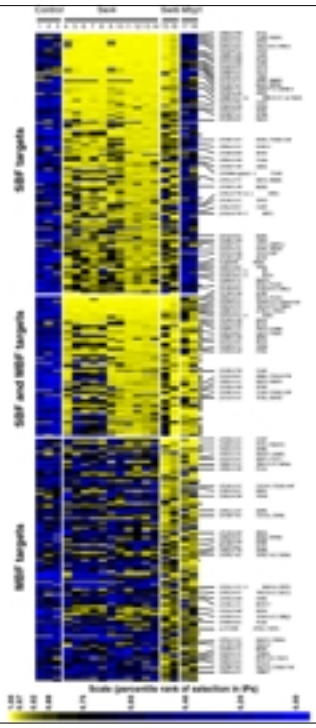


Reading cis-Regulatory Code What do we need to know?

Input to system

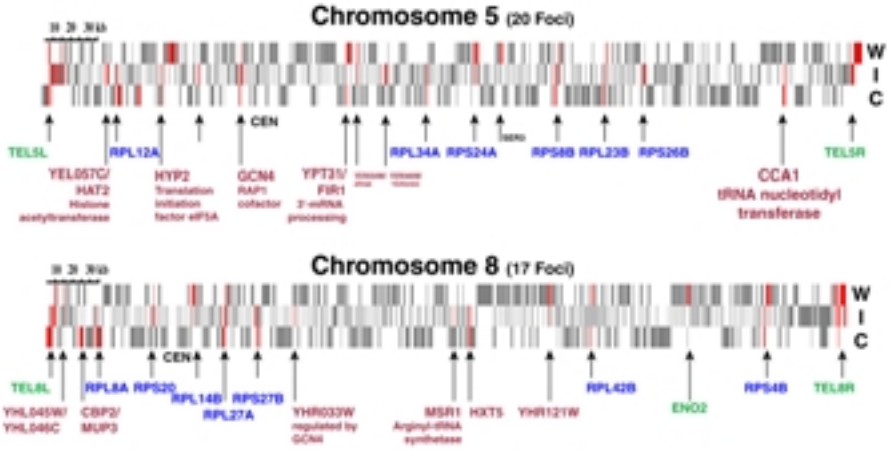
- cis-DNA sequence
- *in vitro* binding affinities of transcription factors
- *in vivo* binding distribution of transcription factors
- Evolutionarily conserved non-coding sequences





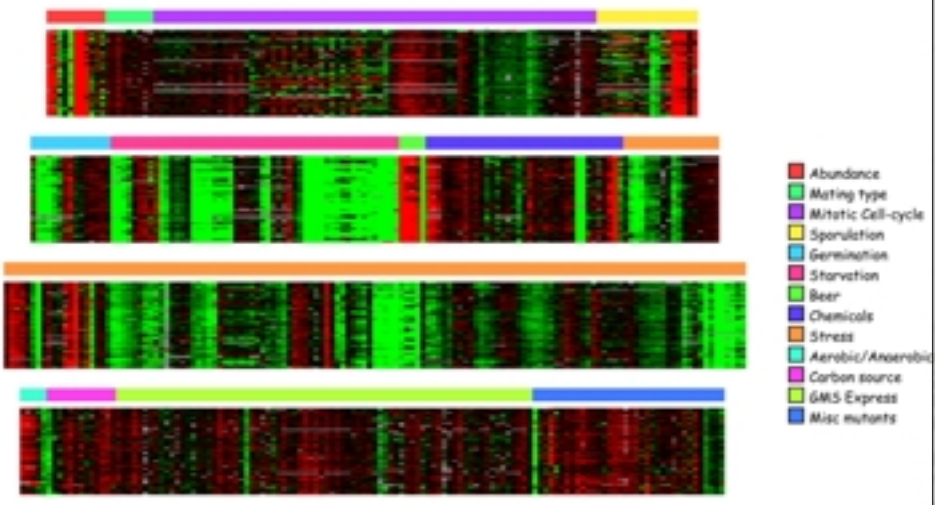
Genomic DNA segments
that consistently
co-immunoprecipitate
with SBF or MBF subunits

RAP1 is Localized to Telomeres and 263 Internal Foci, Potentially Affecting the Regulation of 358 Genes

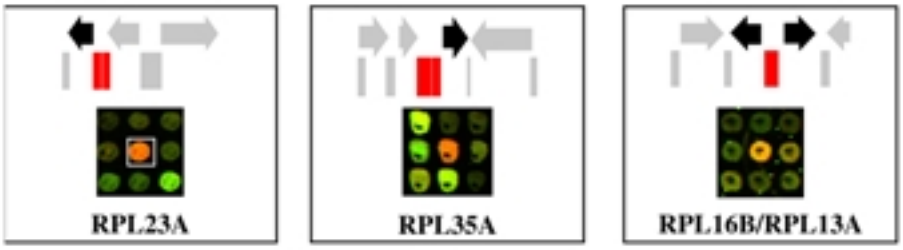


Cytoplasmic Ribosome Cluster

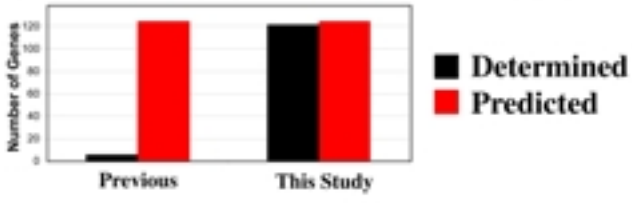
Cluster contains virtually all subunits of cytoplasmic ribosome



RAP1 Regulates the Ribosomal Protein Genes



90% (124 of 137) of Ribosomal Promoters were Selected
98% (121 of 124) of Predicted Targets were Selected



The RAP1 Binding Site Can Be Determined from IP Data Alone

Published RAP1 Consensus Sites

#1	RTRCACCCANNCMCC	Oligo Selection/Amplification
#2	WACAYCCRTACATY	In Silico w/ Ribosomal Promoters
#3	RMAYCCRMNCAYY	Monomer Binding in Vitro
#4	RMACCCANNCAYY	Original In Vitro/Sequence Data
#5	ACACCCAYACAYYY	Structure/Function Analysis
#6	ACAYYY//ACAYYY	Structure/Function Analysis

RCACCCANNCAYY Overall Consensus

Rap1p Binding Sites Determined by IP Data Alone

	Selected Intergenics only	No Telomerics
Consensus:	ACACCCATACATC	ACACCCATACATC
Degenerate:	ACACCCRTACAYY	ACAYCCRTACAYY